

Claims 6 and 39 have been amended to remove recitation of “electroporation.” While the applicants traverse the rejection of the claims as non-enabled for electroporation, they make this amendment solely to expedite prosecution and allowance of the remaining subject matter.

With regard to *Agrobacterium tumefaciens*-mediated transformation, the Office Action alleged that the Applicants’ statements as to the predictability of *Agrobacterium tumefaciens*-mediated transformation of poinsettia were not deemed probative. But the burden is not on the applicants to establish that the claims are enabled for *Agrobacterium tumefaciens*-mediated transformation. Rather, the applicants’ disclosure is presumptively enabling, and the burden is on the Patent Office to provide some scientifically acceptable reasoning or evidence to the contrary. *In re Marzocchi*, 169 U.S.P.Q. 367, 369-70 (C.C.P.A. 1971).

The only reasoning or evidence of non-enablement proffered by the Patent Office relates to prior art transformation failures employing *Agrobacterium rhizogenes*. As previously demonstrated, however, teachings regarding *Agrobacterium rhizogenes* are not indicative of the results the skilled artisan would expect to achieve with *Agrobacterium tumefaciens*. Having disposed of the argument regarding *Agrobacterium rhizogenes*, there are no remaining reasons or evidence of record as to why the claimed methods are not enabled for *Agrobacterium tumefaciens*-mediated transformation. Accordingly, the applicants respectfully submit that the § 112 rejection for non-enablement is unsupported and request its reconsideration and withdrawal.

#### **Rejection of claims 1-3, 97, 101, 104, 113, and 116 under 35 U.S.C. § 103(a)**

Claims 1-3, 97, 101, 104, 113, and 116 were rejected as obvious over Preil *et al.* taken with Nataraja in light of Lee *et al.* For the reasons presented below, the applicants respectfully traverse.

The essence of the rejection is that it would have been obvious to modify the method of Preil *et al.* to incorporate casein hydrolysate because Nataraja taught casein hydrolysate (Lee *et al.* being relied upon to show that the method of Preil *et al.* inherently produces reddish epidermal calli as recited in the present claims). The applicants traverse this allegation for several reasons.

The art as a whole does not suggest or motivate one skilled in the art to modify the method of Preil *et al.* Preil *et al.* was published in 1994, 20 years after the publication of

Nataraja and, as stated in its first paragraph, provides procedures for *in vitro* culturing of poinsettia available at the time. Presumably Preil *et al.* disclosed the best methods of *in vitro* culturing known. Surely had it been desirable to employ casein hydrolysate as taught by Nataraja in 1974, Preil *et al.* would have incorporated such a teaching in its review 20 years later. Yet such a teaching is conspicuously absent.

Furthermore, casein hydrolysate is but a single compound among many employed by Nataraja, and Nataraja does not distinguish it from any of the other compounds as being particularly desirable for use in *in vitro* culturing of poinsettia. There is simply no motivation provided in the cited art to pluck this particular compound from among the many disclosed by Nataraja for use in the method of Priel *et al.*

Lastly, even were one to combine Nataraja's teaching regarding casein hydrolysate with the teachings of Preil *et al.*, one would still not arrive at the presently claimed invention. Nataraja teaches the use of casein hydrolysate in a medium for culturing de-coated seeds, which enlarged with the subsequent emergence of radicle that swelled with proliferation of the hypocotyls region to yield a mass of yellowish-brown, fleshy callus. By contrast, the presently claimed invention employs casein hydrolysate in an embryo induction medium to subculture reddish epidermal callus to form embryogenic callus. Additionally, Preil *et al.* and Nataraja fail to teach the use of (a)  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in an embryo induction medium, (b) an osmotic pressure increasing agent in a developmental medium, or (c) abscisic acid in a maturation medium. The absence of even one of any of the foregoing elements (let alone all) makes the combination of Preil *et al.* and Nataraja insufficient for establishing obviousness.

In view of the foregoing, the applicants respectfully request reconsideration and withdrawal of this § 103 obviousness rejection.

#### **Rejection of claims 1-3, 6-8, 11-41, 44-45, 47-106, and 108-118 under 35 U.S.C. § 103(a)**


Claims 1-3, 6-8, 11-41, 44-45, 47-106, and 108-118 were rejected as obvious over Miki *et al.* with Preil *et al.* and Nataraja in light of Lee *et al.* This rejection is substantively the same as the previously discussed rejecting with the additional reliance on Miki *et al.* for its teaching of particle bombardment. Yet Miki *et al.* does not cure the deficiencies of Preil *et al.*, Nataraja, and Lee *et al.* detailed above. That is, the combination of Miki *et al.* with Preil *et al.*, Nataraja, and

Lee *et al.* does not provide any teachings that would suggest or motivate one skilled in the art to identify casein hydrolysate among the many compounds employed by Nataraja as desirable for use in the methods of Preil *et al.* Nor does the art taken as a whole provide any teachings regarding (a)  $\text{NH}_4^+$  or  $\text{NO}_3^+$  in an embryo induction medium, (b) an osmotic pressure increasing agent in a developmental medium, or (c) abscisic acid in a maturation medium. Thus, even were one to combine the art as suggested, one would not arrive at the presently claimed method. Consequently, the claimed method cannot be obvious.

In view of the foregoing, the applicants respectfully request reconsideration and withdrawal of this § 103 obviousness rejection.

If there are any questions or comments regarding this Response or application, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,



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Claims Amended May 2001 for Application Serial No. 08/903,944

**Redlined Version of Amended Claims**

6. (Three Times Amended) A method for producing transgenic poinsettia plants, comprising:
- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
  - (c)
    - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene; wherein the vector or vectors are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors; or by microprojectile-mediated delivery of the vector into the callus; ~~or by electroporation;~~
  - (d) culturing said transformed embryogenic callus on selection medium;
  - (e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (f) culturing said transgenic embryos on maturation medium; and

(g) recovering transgenic plants from said transgenic embryos.

39. (Four Times Amended) A method for producing transgenic poinsettia plants, comprising:

- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;
- (b) subculturing embryogenic callus produced on said callus induction medium ~~in~~ to liquid  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  containing embryo induction medium;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) subculturing embryos produced on said embryo induction medium ~~on~~ to maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) culturing epidermal callus produced on said callus induction medium on embryo induction medium to form embryogenic callus;
- (h)
  - (i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene; wherein the vector or vectors are introduced into the incubating embryogenic callus by

co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors; or by microprojectile-mediated delivery of the vector into the callus; ~~or by electroporation;~~

- (i) culturing said transformed embryogenic callus on selection medium;
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (k) culturing said transformed embryos on maturation medium; and
- (l) recovering transgenic plants from said transgenic embryos.

103. (Amended) A method for producing transgenic poinsettia plants comprising the steps of:

- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
- (b) subculturing embryogenic callus produced on said callus induction medium ~~in~~ to liquid embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$ ;
- (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
- (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
- (e) subculturing embryos produced on said embryo induction medium ~~on~~ to maturation medium;
- (f) culturing said embryos on callus induction medium;
- (g) culturing embryogenic callus produced on said callus induction medium on embryo induction medium to form embryogenic callus containing embryos;

- (h)
  - (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;
- (i) culturing said transformed embryogenic callus on selection medium;
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (k) culturing said transformed embryos on maturation medium; and
- (l) recovering transgenic plants from said transgenic embryos.